

In the Specification:

Please replace the first paragraph on page 1 under the title with the following:

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This application is a divisional of co-pending U.S. Patent Application No. 07/983,647, filed December 1, 1992; which is a continuation-in-part co-pending U.S. Patent Application Serial Number 07/553,759, filed July 13, 1990; which is a continuation-in-part of U.S. Patent Application Serial Number 07/498,809 filed March 23, 1990 (abandoned); which is a continuation-in-part of U.S. Patent Application Serial Number 07/379,076, filed July 13, 1989 (abandoned); which is a continuation-in-part of co-pending U.S. Patent Application Serial Number 07/160,416, filed February 25, 1988 (abandoned). Each of these predecessor applications and all references cited herein are incorporated by reference in their entirety.

Please replace fourth paragraph on page 10, from line 33 to page 11, line 9 with the following:

AS  
A further aspect of the present invention comprises a synthetic transcription unit for use in a cDNA expression vector, comprising a chimeric promoter composed of human cytomegalovirus AD169 immediate early enhancer sequences fused to HIV LTR -60 to +80 sequences. The small size and particular arrangement of the sequences of the cDNA expression vector of the present invention allow highly efficient replication in host mammalian tissue culture cells, such as COS cells. Moreover, this vector employs a polylinker containing two inverted BstXI sites separated by a short replaceable DNA segment, which allows the use of a very efficient oligonucleotide-based cDNA insertion strategy.

Please replace third paragraph on page 12 from line 22 to line 32 with the following:

Ab  
The purified genes and proteins of the present invention are useful for immunodiagnostic and immunotherapeutic applications, including the diagnosis and treatment of immune-mediated diseases, infections, and disorders in animals, including humans. They can also be used to identify, isolate and purify other antibodies and antigens. Such diagnostic and therapeutic uses comprise yet another aspect of the present invention. Moreover, the substantially pure proteins of the present invention may be

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A6

prepared as medicaments or pharmaceutical compositions for therapeutic administration. The present invention further relates to such medicaments and compositions.

Please replace the first paragraph on page 13, from line 2 to line 15 with the following:

**Figures 1A-1B.** Nucleotide sequence of expression vector piH3 (SEQ ID NO:1)

A7  
Nucleotides 1-589 are derived from pMB1 origin (pBR322 ori); nucleotides 590-597 are derived from the SacII linker (ACCGCGT); nucleotides 598-799 are derived from the synthetic tyrosine suppressor tRNA gene (supF gene); nucleotides 800-947 are derived from a remnant of the ASV LTR fragment (PvuII to MluI); nucleotides 948-1500 are derived from the human cytomegalovirus AD169 enhancer; nucleotides 1501-1650 are derived from HIV TATA and tat-responsive elements; nucleotides 1651-1716 are derived from the piLNxAN polylinker (HindIII to Xba); nucleotides 1717-2569 are derived from pSV to splice and poly-Adenylation signals; nucleotides 2570-2917 are derived from the SV40 origin of replication (PvuII to (HindIII); and nucleotides 2918-2922 are derived from piVX, remnant of R1 site from polylinker.

Please replace the second paragraph on page 13, from line 16 to line 22 with the following:

**Figures 2A-2B.** Nucleotide sequence of the CD2cDNA insert (SEQ ID NO:2)

A8  
Nucleotide numbering is given in parentheses at right, amino acid numbering, left. Locations of the potential sites for addition of asparagine-linked carbohydrate (CHO) are shown, as well as the predicted transmembrane (TM) sequence. The amino acid sequence is numbered from the projected cleavage site of the secretory signal sequence.

Please replace the first paragraph on page 14, from line 1 to line 9 with the following:

**Figures 4A-4B.** Nucleotide sequence and corresponding amino acid sequence of the LFA-3 antigen (SEQ ID NO:4)

WOP cells transfected with a clone encoding the LFA-3 antigen were detected by indirect immunofluorescence, amplified and sequenced. Figure 4A shows the 874 base pair insert containing an open reading frame of 237 residues originating at a methionine codon, and terminating in a series of hydrophobic residues. Hydrophobic and hydrophilic regions within this open reading frame are shown in Figure 4B.

Please replace the third paragraph on page 14, from line 14 to line 21 with the following:

**Figures 6A-6D.** Nucleotide sequence of the piH3M vector (SEQ ID NO:6)

There are 7 segments. Residues 1-587 are from the pBR322 origin of replication, 588-1182 from the M13 origin, 1183-1384 from the supF gene, 1385-2238 are from the chimeric cytomegalovirus/human immunodeficiency virus promoter, 2239-2647 are from the replaceable fragment, 2648-3547 from plasmid pSV2 (splice and polyadenylation signals), and 3548-3900 from the SV40 virus origin.

Please replace the fourth paragraph on page 14, from line 22 to line 28 with the following:

**Figures 7A-7B.** Nucleotide sequence of the CD28 cDNA (SEQ ID NO:7)

Nucleotide numbering is given in parentheses at right, amino acid numbering, center and left. Location of the potential sites for addition of asparagine-linked carbohydrate (CHO) are shown, as well as the predicted transmembrane (TM) sequence. The amino acid sequence is numbered from the projected cleavage site of the secretory signal sequence.

Please replace the fifth paragraph on page 14, from line 29 to page 15, line 3 with the following:

**Figures 8A-8B.** Nucleotide sequence of the CD7 cDNA insert (SEQ ID NO:9)

A12  
Nucleotide numbering is given in parentheses at right. Splice donor and acceptor sites indicated by (/). The location of the potential sites for addition of asparagine-linked carbohydrate (CHO) are shown, the potential fatty acid esterification site is denoted (\*), and the predicted transmembrane domain (TM) is underlined. Nucleotide sequences potentially involved in hairpin formation are denoted by (..). The presumed polyadenylation signal is underlined.

Please replace the second paragraph on page 15, from line 4 to line 11 with the following:

**Figures 9A-9B.** Nucleotide sequence of the CDw32 cDNA (SEQ ID NO:10)

A13  
Nucleotide number is given in the parenthesis at right, amino acid numbering, center and left. Locations of the potential sites for addition of asparagine-linked carbohydrate (CHO) are shown, as well as the predicted transmembrane (TM) sequence. The amino acid sequence is numbered from the projected cleavage site of the secretory signal sequence. Cysteine residues are underscored with asterisks.

Please replace the third paragraph on page 15, from line 12 to line 18 with the following:

**Figures 10A-10C.** Sequence of the CD20.4 cDNA (SEQ ID NO:11)

A14  
Figures 10A - 10B. The sites of potential N-linked glycosylation are denoted by the symbol -CHO-; the hydrophobic regions are underscored. The site of the poly(A)<sup>+</sup> tail in clone CD20.6 is denoted by an asterisk.

Figure 10C. Presents a hydrophobicity profile of the amino acid sequence in Figures 10A-10B.

Please replace the fourth paragraph on page 15, from line 19 to line 29 with the following:

**Figures 11A-11C.**      Sequence of ICAM-1 (SEQ ID NO:13)

A15  
Complete nucleotide sequence of ICAM-1 cDNA insert and predicted protein sequence. Nucleotide numbering is at left, amino acid numbering, center. The RGE motif at position 128 is underlined, the potential N-linked glycosylation sites are indicated by -CHO- and the transmembrane domain by -TM-. The amino acid sequence is numbered from the projected cleavage site of the signal peptide. Sequencing was by dideoxy-chain termination (Sanger, F., et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977)), using a combination of subclones, and specific oligonucleotides.

Please replace the fifth paragraph on page 15, line 30 with the following:

A16  
**Figures 12A-12B.**      Nucleotide sequence of CD19 (SEQ ID NO:15)

Please replace the sixth paragraph on page 15, line 31 with the following:

A17  
**Figures 13A-13B.**      Nucleotide sequence of CD20 (SEQ ID NO:16)

Please replace the first paragraph on page 16, line 1 with the following:

A18  
**Figure 14A-14B.**      Nucleotide sequence of CDw32a (SEQ ID NO:17)

Please replace the second paragraph on page 16, line 2 with the following:

A19  
**Figures 15A-15B.**      Nucleotide sequence of CDw32b (SEQ ID NO:18)

Please replace the third paragraph on page 16, line 3 with the following:

A20  
**Figure 16.**      Nucleotide sequence of CD40 (SEQ ID NO:19)

Please replace the second paragraph on page 18 from line 5 to line 30 with the following:

A21  
The guanidium thiocyanate/CsCl method of isolating total RNA is preferred. More preferred is a guanidium thiocyanate/LiCl variant of the GuSCN/CsCl method, which has added capacity and speed. Briefly, for each ml of mix desired, 0.5g GuSCN are dissolved in 0.58 ml of 25% LiCl (stock filtered through 0.45 micron filter) and 20  $\mu$ l of mercaptoethanol is added. Cells are spun out and the pellet is dispersed on walls by flicking, add 1 ml of solution up to  $5 \times 10^7$  cells. The resulting combination is sheared by polytron until nonviscous. For small scale preps (less than  $10^8$  cells) layer 2 ml of sheared mix on 1.5 ml of 5.7M CsCl (RNase free; 1.26g CsCl added to every ml 10 mM EDTA pH 8), overlay with RNase-free water and spin SW55 50k rpm 2h. For large scale preps, layer 25 ml on 12 ml CsCl in a SW28 tube, overlay, and spin 24k rpm 8h. Aspirate contents carefully with a sterile pasteur pipet connected to a vacuum flask. Once past the CsCl interface, scratch a band around the tube with the pipet tip to prevent the layer on the wall of the tube from creeping down. The remaining CsCl solution is aspirated. The pellets are taken up in water (do not try to redissolve). 1/10 vol. NaOAc and 3 vol. EtOH are added and the resulting combination is spun. If necessary, the pellet is resuspended in water (e.g., at 70°). Adjust concentration to 1 mg/ml and freeze. Small RNA (e.g. 5S) does not come down. For small amounts of cells, scale down volumes and overlay GuSCN with RNase-free water on gradient (precipitation is inefficient when RNA is dilute).

Please replace the third paragraph on page 18 from line 32 to page 19, line 17 with the following:

A22  
Next, polyA<sup>+</sup> RNA may be prepared, preferably by the oligo dT selection method. Briefly, a disposable polypropylene column is prepared by washing with 5M NaOH and then rinsing with RNase-free water. For each milligram total RNA about 0.3 ml (final packed bed) oligo dT cellulose is used. Oligo dT cellulose is prepared by resuspending about 0.5 ml of dry powder in 1 ml of 0.1M NaOH and transferring it into the column, or by percolating 0.1 NaOH through a previously used column (columns can be reused many times). This is washed with several column volumes of RNase-free water, until pH is neutral, and rinsed with 2-3 ml of loading buffer. The column bed is then removed into a sterile 15 ml tube using 4-6 ml of loading buffer. The total RNA was heated to 70°C for 2-3 min., LiCl from RNase-free stock is added (to 0.5M), and combined with oligo dT cellulose in a 15 ml tube. This is followed by vortexing or agitation for 10 min. The result is poured into a column and washed with 3 ml loading

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A22  
buffer and then 3 ml of middle wash buffer. mRNA is eluted directly into an SW55 tube with 1.5 ml of 2 mM EDTA, 0.1% SDS; the first two or three drops are discarded.

Please replace the second paragraph on page 19, from line 18 to line 24 with the following:

A23  
Eluted mRNA is precipitated by adding 1/10 vol. 3M NaOAc and filling the tube with EtOH. This is then mixed, chilled for 30 minutes at -20°C, and spun at 50k rpm at 5°C for 30 min. The EtOH is poured off and the tube is air dried. The mRNA pellet is resuspended in 50-100 µl of RNase-free water. Approximately 5 µl is melted at 70°C in MOPS/EDTA/ formaldehyde and run on an RNase-free 1% agarose gel to check quality.

Please replace the fourth paragraph on page 19, from line 29 to page 20, line 4 with the following:

A24  
a. First Strand. 4 µg of mRNA and heated to about 100°C in a microfuge tube for 30 seconds and quenched on ice. The volume is adjusted to 70 µl with RNase-free water. The following are added: 20 µl of RT1 buffer, 2 µl of RNase inhibitor (Boehringer 36 µl/µl), 1 µl of 5 µg/µl of oligo dT (Collaborative Research), 2.5 µl of 20 mM dXTP's (ultrapure), 1 µl of 1 M DTT and 4 l of RT-LX (Life Science, 24 U/µl). The resulting combination is incubated at 42°C for 40 min. It is heated to inactivate (70°C 10 min).

Please replace the second paragraph on page 20, from line 5 to line 12 with the following:

A25  
b. Second Strand. 320 µl of RNase free water, 80 µl of RT2 buffer, 5 µl of DNA Polymerase I (Boehringer, 5 µl/µl), 2 µl RNase H (BRL 2 U/µl). Incubate at 15°C for 1 hr and 22°C for 1 hr. Add 20 µl of 0.5M EDTA pH 8.0, phenol extract and EtOH precipitate by adding NaCl to 0.5M, linear polyacrylamide (carrier) to 20 µg/ml, and filling tube with EtOH. Spin 2-3 minutes in microfuge, remove, vortex to dislodge precipitate high up on wall of tube, and respin 1 minute.

Please replace the third paragraph on page 20, from line 13 to line 20 with the following:

A26  
c. Adaptors. Resuspend precipitated cDNA in 240  $\mu$ l of TE (10/1). Add 30  $\mu$ l of 10x low salt buffer, 30  $\mu$ l of 10X low salt buffer, 30  $\mu$ l of 10X ligation additions, 3  $\mu$ l (2.4 $\mu$ g) of kinased 12-mer adaptor, 2  $\mu$ l (1.6 $\mu$ g) of kinased 8-mer adaptor, and 1  $\mu$ l of T4 DNA ligase (BioLabs, 400  $\mu$ /ml, or Boehringer, 1 Weiss unit/ml). Incubate at 15°C overnight. Phenol extract and EtOH precipitate as above (no extra carrier now needed), and resuspend in 100  $\mu$ l of TE.

Please replace the fifth paragraph on page 20, from line 28 to page 21, line 22 with the following:

A27  
Prepare a 20% KOAc, 2 mM EDTA, 1  $\mu$ g/ml EthBr solution and a 5% KOAc, 2 mM EDTA, 1  $\mu$ g/ml EthBr solution. Add 2.6 ml of 20% KOAc solution to back chamber of a small gradient maker. Remove air bubble from tube connecting the two chambers by allowing solution to flow into the front chamber and then tilt back. Close passage between chambers, and add 2.5 ml of the 5% solution to the front chamber. If there is liquid in the tubing from a previous run, allow the 5% solution to run just to the end of the tubing, and then return to chamber. Place the apparatus on a stirplate, set the stir bar moving as fast as possible, open the stopcock connecting the two chambers and then open the front stopcock. Fill a polyallomer SW55 tube from the bottom with the KOAc solution. Overlay the gradient with 100  $\mu$ l of cDNA solution. Prepare a balance tube and spin the gradient for 3 hrs at 50k rpm at 22°C. To collect fractions from the gradient, pierce the SW55 tube with a butterfly infusion set (with the luer hub clipped off) close to the bottom of the tube and collect three 0.5 ml fractions and then 6 0.25 ml fractions into microfuge tubes (about 22 and 11 drops respectively). EtOH precipitate the fractions by adding linear polyacrylamide to 20  $\mu$ g/ml and filling the tube to the top with EtOH. After cooling tubes, spin them in a microfuge for 3 min. Vortex and respin 1 min. Rinse pellets with 70% EtOH (respin). Do not dry to completion. Resuspend each 0.25 ml fraction in 10  $\mu$ l of TE. Run 1  $\mu$ l on a 1% agarose minigel. Pool the first three fractions, and those of the last six which contain no material smaller than 1 kb.



Please replace the second paragraph on page 21, from line 23 to page 22, line 5 with the following:

A28  
Suppressor tRNA plasmids may be propagated by known methods. In a preferred method according to the present invention, supF plasmids can be selected in nonsuppressing hosts containing a second plasmid, p3, which contains amber mutated ampicillin and tetracycline drug resistance elements (Seed, 1983). The p3 plasmid is derived from PR1, is 57 kb in length, and is a stably maintained, single copy episome. The ampicillin resistance of this plasmid reverts at a high rate, so that amp<sup>r</sup> plasmids usually cannot be used in p3-containing strains. Selection for tet resistance alone is almost as good as selection for amp<sup>r</sup>+tet resistance. However, spontaneous appearance of chromosomal suppressor tRNA mutations presents an unavoidable background (frequency about 10<sup>-9</sup>) in this system. Colonies arising from spontaneous suppressor mutations are usually bigger than colonies arising from plasmid transformation. Suppressor plasmids typically are selected for in LB medium containing amp at 12.5 µg/ml and tet at 7.5 µg/ml. For large plasmid preps, M9 casamino acids medium containing glycerol (0.8%) may be used as a carbon source, and the bacteria grown to saturation.

Please replace the first paragraph on page 23, from line 1 to line 17 with the following:

A29  
The vector may be prepared for cloning by known methods. A preferred method begins with cutting 20 µg of vector in a 200 µl reaction with 100 units of BstXI (New York Biolabs), cutting at 50°C overnight in a well-thermostatted water bath (i.e., circulating water bath). Prepare 2 KOAc 5-20% gradients in SW55 tubes as described above. Add 100 µl of the digested vector to each tube and run for 3 hrs, 50K rpm at 22°C. Examine the tube under 300 nm UV light. The desired band will have migrated 2/3 of the length of the tube. Forward trailing of the band means the gradient is overloaded. Remove the band with a 1 ml syringe and 20 gauge needle. Add linear polyacrylamide and precipitate the plasmid by adding 3 volumes of EtOH. Resuspend in 50 µl of TE. Set up ligations using a constant amount of vector and increasing amounts of cDNAs. On the basis of these trial ligations, set up large scale ligation, which can be accomplished by known methods. Usually the entire cDNA prep requires 1-2 µg of cut vector.

Please replace the second paragraph on page 23, from line 18 to line 24 with the following:

A30  
Adaptors may be prepared by known methods, but it is preferred to resuspend crude adaptors at a concentration of 1  $\mu\text{g}/\mu\text{l}$ , add  $\text{MgSO}_4$  to 10 mM, and precipitate by adding 5 volumes of EtOH. Rinse with 70% EtOH and resuspend in TE at a concentration of 1  $\mu\text{g}/\mu\text{l}$ . To kinase take 25  $\mu\text{l}$  of resuspended adaptors, add 3  $\mu\text{l}$  of 10X kinasing buffer and 20 units of kinase; incubate 37°C overnight.

Please replace the second paragraph on page 26, from line 15 to page 27, line 24 with the following:

A31  
If spheroplast fusion is employed, a preferred method is the following variant based on Sandri-Goldrin et al., Mol. Cell Bio. 1:743-752 (1981). Briefly, for example, a set of six fusions requires 100 ml of cells in broth. Grow cells containing amplifiable plasmid to OD 600=0.5 in LB. Add spectinomycin to 100  $\mu\text{g}/\text{ml}$  (or chloramphenicol to 150  $\mu\text{g}/\text{ml}$ ). Continue incubation at 37°C with shaking for 10-16 hours. (Cells begin to lyse with prolonged incubation in spectinomycin or chloramphenicol medium). Spin down 100 ml of culture (JA14/GSA rotor, 250 ml bottle) 5 min. at 10,000 rpm. Drain well, resuspend pellet in bottle with 5 ml cold 20% sucrose, 50 mM Tris-HCL pH 8.0. Incubate on ice 5 min. Add 2 ml cold 0.25M EDTA pH 8.0, incubate 5 min. at 37°C (waterbath). Place on ice, check percent conversion to spheroplasts by microscopy. In flow hood, slowly add 20 ml of cold DME/10% sucrose/10 mM  $\text{MgCl}_2$  (dropwise, ca. 2 drops per second). Remove media from cells plated the day before in 6 cm dishes (50% confluent). Add 5 ml of spheroplast suspension to each dish. Place dishes on top of tube carriers in swinging bucket centrifuge. Up to 6 dishes can be comfortably prepared at once. Dishes can be stacked on top of each other, but 3 in a stack is not advisable as the spheroplast layer on the top dish is often torn or detached after centrifugation. Spin at 1000xg 10 min. Force is calculated on the basis of the radius to the bottom plate. Aspirate fluid from dishes carefully. Pipet 1.5-2 ml 50% (w/w) PEG 1450 (or PEG 1000)/50% DME (no serum) into the center of the dish. If necessary, sweep the pipet tip around to ensure that the PEG spreads evenly and radially across the whole dish. After PEG has been added to the last dish, prop all of the dishes up on their lids so that the PEG solution collects at the bottom. Aspirate the PEG. The thin layer of PEG that remains on the cells is sufficient to promote fusion; the layer remaining is easier to wash off, and better cell viability can be obtained, than if the bulk of the PEG is left behind. After 90 to 120 seconds (PEG 1000) or 120 to 150 seconds (PEG 1450) of contact with the PEG solution, pipet 1.5 ml of DME (no serum) into the center of the dish. The PEG

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layer will be swept radially by the DME. Tilt the dishes and aspirate. Repeat the DME wash. Add 3 ml of DME/10% serum containing 15  $\mu$ g/ml gentamicin sulfate. Incubate 4-6 hours in incubator. Remove media and remaining bacterial suspension, add more media and incubate 2-3 days. Extensive washing of the cell layer to remove PEG tends to remove many of the cells without any substantial benefit. If the cells are allowed to sit in the second DME wash for a few minutes, most of the spheroplast layer will come up spontaneously; however it is preferred to wash briefly and allow the layer to come off in the complete medium at 37°C.

Please replace the fifth paragraph on page 29, from line 29 to page 30, line 4 with the following:

A32

a. Antibody-coated dishes. Bacteriological 60 mm plates, Falcon 1007 or equivalent, or 10 cm dishes such as Fisher 8-757-12 may be used. Sheep anti-mouse affinity purified antibody (from, for example, Cooper BioMedical (Cappell)) is diluted to 10  $\mu$ g/ml in 50 mM Tris HCl, pH 9.5. Add 3 ml per 6 cm dish, or 10 ml per 10 cm dish. Let sit ca. 1.5 hrs., remove to next dish 1.5 hrs., then to 3rd dish. Wash plates 3x with 0.15 M NaCl (a wash bottle is convenient for this), incubate with 3 ml 1 mg/ml BSA in PBS overnight, aspirate and freeze.

Please replace the third paragraph on page 30, from line 22 to page 31, line 2 with the following:

A33

c. Hirt Supernatant. A preferred variant of the method of Hirt, J. Molec. Biol. 26:365-369 (1967), is as follows: Add 0.4 ml 0.6% SDS, 10 mM EDTA to panned plate. Let sit 20 minutes (can be as little as 1 min. if there are practically no cells on the plate). Pipet viscous mixture into microfuge tube. Add 0.1 ml 5M NaCl, mix, put on ice at least 5 hrs. Keeping the mixture as cold as possible seems to improve the quality of the Hirt. Spin 4 min., remove supernatant carefully, phenol extract (twice if the first interface is not clean), add 10  $\mu$ g linear polyacrylamide (or other carrier), fill tube to top with EtOH, precipitate, and resuspend in 0.1 ml. Add 3 volumes EtOH/NaOAc, reprecipitate and resuspend in 0.1 ml. Transform into MC1061/p3, preferably using the high efficiency protocol hereinafter described. If the DNA volume exceeds 2% of the competent cell aliquot, the transformation efficiency will suffer. 5% gives the same number of colonies as 2.5% (efficiency is halved).

Please replace the second paragraph on page 31, from line 3 to line 18 with the following:

A34  
It is preferred for this aspect of the present invention to use "blockers" in the incubation medium. Blockers assure that non-specific proteins, proteases, or antibodies present do not cross-link with or destroy the antibodies present on the substrate or on the host cell surface, to yield false positive or false negative results. Selection of blockers can substantially improve the specificity of the immunoselection step of the present invention. A number of non-specific monoclonal antibodies, for example, of the same class or subclass (isotype) as those used in the immunoselection step (e.g., IgG<sub>1</sub>, IgG<sub>2A</sub>, IgGm, etc.) can be used as blockers. Blocker concentration (normally 1-100 µg/µl) is important to maintain the proper sensitivity yet inhibit unwanted interference. Those of skill also will recognize that the buffer system used for incubation may be selected to optimize blocking action and decrease non-specific binding.

Please replace the fourth paragraph on page 33, from line 27 to page 34, line 6 with the following:

A35  
Insertion of cDNA into the vectors of the present invention can occur, for example, by homopolymeric tailing with terminal transferase. However, homopolymeric tracts located 5' to cDNA inserts may inhibit in vitro and in vivo expression. Thus, preferred for purposes of the present invention is the use of inverted identical cleavage sites separated by a short replaceable DNA segment. Such inverted identical cleavage sites, preferably employing the BstXI restriction endonuclease, may be used in parallel with cDNA synthetic oligonucleotides, giving the same termini as the replaceable segment of the vector. In this manner, the cDNA cannot ligate to itself, but can ligate to the vector. This allows the most efficient use of both cDNA and vector.

Please replace the third paragraph on page 39, from line 11 to line 23 with the following:

A36  
A COS cell expression vector was constructed from piSV (Little et al., Mol. Biol. Med. 1:473-488 (1983)) by inserting a synthetic transcription unit between the suppressor tRNA gene and the SV40 origin. The transcription unit consisted of a chimeric promoter composed of human cytomegalovirus AD169 immediately early enhancer sequences fused to the HIV LTR -67 to +80 sequences. Immediately downstream from the LTR +80 sequence was inserted a polylinker containing two BstXI sites separated

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by a 350bp stuffer; the BstXI sites were flanked by XbaI sites, which could also be used to excise the insert. Downstream from the polylinker were placed the SV40 small t antigen splice and early region polyadenylation signals derived from pSV2. The nucleotide sequence of the vector is shown in Figures 1A-1B.

Please replace the fourth paragraph on page 39, from line 24 to page 40, line 4 with the following:

cDNA library construction

A37  
RNA was prepared from HPB-ALL cells by the guanidinium thiocyanate/CsCl method, as described above. PolyA<sup>+</sup> RNA was prepared from total RNA by oligo dT selection. Maniatis et al, Molecular Cloning: A Laboratory Manual, supra. cDNA was synthesized by the method of Gubler and Hoffman (Gene 25:263-269 (1982)). BstXI adaptors were ligated to the cDNA, and the reaction products fractionated by centrifugation through a 5 ml-20% potassium acetate gradient containing 1 mM EDTA for 3 hours at 50k rpm in a SW55 rotor. 0.5 ml fractions were collected manually through a syringe needle or butterfly inserted just above the curve of the tube. Individual fractions were ethanol-precipitated after addition of linear polyacrylamide (Strauss and Varshavsky, Cell 37:889-901 (1984)) to 20 µg/ml. Fractions containing cDNA larger than 700bp were pooled and ligated to gradient purified BstXI digested pIH3 vector.

Please replace the second paragraph on page 40, from line 5 to line 30 with the following:

A38  
The ligated DNA was transformed into E. coli MC1061/p3 made competent by the following protocol: The desired strain was streaked out on an LB plate. The next day a single colony was inoculated into 20 ml TYM broth (recipes below) in a 250 ml flask. The cells were grown to midlog phase (OD<sub>600</sub> about 0.2-0.8), poured into a 2L flask containing 100 ml TYM, and vigorously agitated until cells grew to 0.5-0.9 OD, then diluted again to 500 ml in the same vessel. When the cells grew to OD<sub>600</sub> 0.6, the flask was placed in ice-water, and shaken gently to assure rapid cooling. When the culture was cool, it was spun at 4.2k rpm for 15 minutes (J6). The supernatant was poured off and the pellet resuspended in about 100 ml cold Tfb I (below) by gentle shaking on ice. Thereafter, it was respun in the same bottle at 4.2k rpm for 8 minutes (J6). The supernatant was poured off and the pellet

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A38

resuspended in 20 ml cold Tfb II by gentle shaking on ice. 0.1 to 0.5 ml aliquots were placed in prechilled microfuge tubes, frozen in liquid nitrogen, and stored at -70°C. For transformation, an aliquot was removed, thawed at room temperature until just melting, and placed on ice. DNA was added, let sit on ice 15-30 minutes, and incubated at 37°C for 5 minutes (6 minutes for 0.5 ml aliquots). Thereafter the DNA-containing suspensions were diluted 1:10 in LB and grown for 90 minutes before plating or applying antibiotic selection. Alternatively, the heat-pulsed transformation mix was plated directly on antibiotic plates onto which a thin (4-5 ml) layer of antibiotic-free LB agar was poured just before plating.

Please replace the second paragraph on page 41, from line 29 to page 42, line 2 with the following:

#### Cell lines and cell culture

A39

COS cell clone M6 cells were propagated in Dulbecco's modified Eagle's medium supplemented with 10% calf serum and gentamycin sulfate at 15 µg/ml (DME/10% calf serum). Cells were split the day before transfection in 6 cm dishes at approximately 1:8 ratio from stock plates kept as dense as possible without overtly affronting the cells. T cell lines were grown in Iscove's modification of Dulbecco's medium (IMDM) containing gentamycin as above, and either NuSerum (Collaborative Research) or fetal bovine serum at 10%.

Please replace the second paragraph on page 42, from line 3 to line 12 with the following:

#### COS cell transfection for immunofluorescence studies

A40

COS cells at 50% confluence in 6 cm dishes were transfected in a volume of 1.5 ml with a cocktail consisting of DME or IMDM medium containing 10% NuSerum (Collaborative Research), 400 µg/ml DEAE Dextran, 10µM chloroquine diphosphate, and 1 µg/ml DNA. After 4 hours at 37°C (or earlier if the cells appeared ill), the transfection mix was removed and the cells were treated with 10% DMSO in PBS for 2 minutes. Sussman and Milman, Cell Biol. 4:1641-1643 (1984). Cells were then returned to DME/10% calf serum for 48 to 72 hours to allow expression.

Please replace the fourth paragraph on page 42, from line 28 to page 43, line 11 with the following:

A41 Northern blot analysis was carried out essentially as described (Maniatis et al., Molecular Cloning, a Laboratory Manual (1982)), except that DMSO was omitted from the loading buffer, denaturation was at 70°C for 5 minutes, and the gel contained 0.6% formaldehyde rather than 6%. The gel was stained in two volumes of water containing 1 µg/ml ethidium bromide, photographed, and transferred to nylon (GeneScreen, DuPont) in the staining liquor. The transferred RNA was irradiated by exposure to a germicidal lamp through Saran Wrap (Church and Gilbert, Proc. Natl. Acad. Sci. USA 8:1991-1995 (1984)) for 5 minutes at a flux (measured at 254 nm) of 0.22mW/cm<sup>2</sup>. Southern blot analysis was carried out by alkaline transfer to nylon (GeneScreen, DuPont) as described by Reed and Mann (Nucl. Acids Res. 13:7207-7221 (1986)). Hybridization probes were prepared by the method of Hu and Messing (Gene 18:271-277 (1982)), and blots were prehybridized in SDS/phosphate buffer (Church and Gilbert, Proc. Natl. Acad. Sci. USA 8:1991-1995 (1984)) containing 10 DNA microgram equivalents of M13 mp19 phage.

Please replace the second paragraph on page 43, from line 12 to line 21 with the following:

#### Erythrocyte Rosetting

A42 Erythrocytes were prepared from whole blood by three centrifugations in PBS. COS cells were transfected in 6 cm dishes with CD2 or other surface antigen expression clones by the DEAE method. 48 to 72 hours posttransfection, the medium was aspirated and 2 ml of PBS/5% FDS/azide was added to each plate, followed by 0.4 ml of the appropriate erythrocyte samples as 20% suspensions in PBS. After 1 hour at room temperature, the nonadherent erythrocytes were gently washed off, and the plates were examined.

Please replace the fourth paragraph on page 44, from line 26 to page 45, line 7 with the following:

#### cDNA sequence analysis

A43 The CD2 cDNA insert was subcloned into M13 mp19 (Vieira and Messing, Gene 19:259-268 (1982)) in both orientations, and the sequence determined by the dideoxynucleotide method (Figures 2A

Cont  
A43

and 2B). Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467 (1977). An open reading frame was observed to extend 360 residues from an ATG triplet satisfying the consensus criteria of Kozak (Microbiol. Rev.: 1-47:45 (1983)) for translational initiation codons (Figures 1A and 1B). The predicted amino acid sequence evokes an integral membrane protein with a single membrane spanning hydrophobic anchor terminating in a rather large intracytoplasmic domain. Comparison of the N-terminal amino sequence with the matrix of signal sequence residue frequencies constructed by von Heijne (Nucl. Acids Res. 14:4683-4690 (1986)) suggests that mature CD2 peptide is formed by cleavage of a precursor peptide between the 19th (Ser) and 20th (Lys) residues.

Please replace the second paragraph on page 47, from line 16 to line 30 with the following:

COS cells expressing CD2 form rosettes with sheep erythrocytes

A44

COS cells transfected with the CD2 expression clone were treated for 1 hour with purified MT910 (IgG, kappa) anti-CD2 antibody (Rieber et al., Leukocyte Typing II, Vol. I, pp. 233-242 (1986)) at a concentration of 1 µg/ml, or with purified MB40.5 (IgG1, kappa; Kawata et al., J. Exp. Med. 160:633-651 (1984)) antibody at the same concentration. MB40.5 recognizes a monomorphic HLA-ABC determinant and cross-reacts with African Green Monkey histocompatibility antigens; it was chosen because it represents an isotype-matched antibody recognizing a surface antigen of approximately the same abundance as the CD2 antigen expressed by transfected cells. Sheep erythrocyte rosettes were observed in the presence of MB40.5, but not of MT910. Rosette inhibition was also observed with OKT11 antibody, and not with various other control antibodies.

Please replace the fourth paragraph on page 51, from line 22 to line 34 with the following:

A45

A clone encoding the LFA-3 antigen was identified by indirect immunofluorescence of transfected WOP cells, amplified and sequenced (Figure 4A). Within the 874 bp insert, an open reading frame of 237 residues originates at a methionine codon closely corresponding to the consensus sequence suggested by Kozak, Microbiol. Rev. 47:1-45 (1983). The reading frame terminates in a series of hydrophobic residues lacking the characteristic basic anchoring residues of internal membrane proteins, but sharing features with known phosphatidylinositol-linked superficial membrane proteins. The



Cont  
A45

features include clustered serine or threonine residues in a hydrophilic region immediately preceding the hydrophobic domain, and the presence of serines and threonines in the hydrophobic portion.

Please replace the fourth paragraph on page 53, from line 20, to page 54, line 11 with the following:

#### Preparation of cDNA Libraries

A46

Poly(A)+ RNA was prepared from the human T-cell tumor line HPB-ALL by oligo(dT) cellulose chromatography of total RNA isolated by the guanidinium thiocyanate method (Chirgwin, J.M. et al., Biochemistry 18:5294-5299 (1979)). cDNA was prepared by a protocol based on the method of Gubler and Hoffman (Gubler, U. et al., Gene 25:263-269 (1982)). 4 µg of mRNA was heated to approximately 100°C in a 1.5 ml centrifuge tube for 30 seconds, quenched on ice, and the volume adjusted to 70 µl with RNase-free water. To this were added 20 µl of buffer (0.25 M Tris pH 8.8 (8.2 at 42°C), 0.25 M KCl, 30 mM MgCl<sub>2</sub>), 2 µl of RNase inhibitor (Boehringer 36 µl/µl), 1 µl of 1M DTT, 1 µl of 5 µg/µl of oligo dT (Collaborative Research), 2 µl of 25 mM each deoxynucleoside triphosphate (US Biochemicals), and 4 µl of reverse transcriptase (Life Sciences, 24 µl/µl). After 40 minutes at 42°C, the reaction was terminated by heating to 70°C for 10 minutes. To the reaction mix was then added 320 µl of RNase free water, 80 µl of buffer (0.1 M Tris pH 7.5, 25 mM MgCl<sub>2</sub>, 0.5 M KCl, 0.25 mg/ml BSA, and 50 mM DTT), 25 units of DNA Polymerase I (Boehringer), and 4 units of RNase H (BRL). After 1 hour at 15°C and 1 hour at 22°C, 20 µl of 0.5M EDTA pH 8.0 were added, the reaction mixture was extracted with phenol, NaCl was added to 0.5 M, linear polyacrylamide (carrier; Strauss, F. et al., Cell 37:889-901 (1984)) was added to 20 µg/ml, and the tube was filled with ethanol. After centrifugation for 2-3 minutes at 12,000 x g, the tube was removed, vortexed to dislodge precipitate spread on the wall of the tube, and respun for 1 minute.

Please replace the second paragraph on page 54, from line 12 to line 20 with the following:

A47

Unpurified oligonucleotides having the sequence CTCTAAAG and CTTTAGAGCACA (SEQ ID NO:37) were dissolved at a concentration of 1 mg/ml, MgSO<sub>4</sub> was added to 10 mM, and the DNA precipitated by adding 5 volumes of EtOH. The pellet was rinsed with 70% ETOH and resuspended in TE at a concentration of 1 mg/ml. 25 µl of the resuspended oligonucleotides were phosphorylated by the

Cont  
A47  
addition of 3  $\mu$ l of buffer (0.5 M Tris pH 7.5, 10 mM ATP, 20 mM DTT, mM spermidine, 1 mg/ml BSA, and 10 mM  $MgCl_2$ ) and 20 units of polynucleotide kinase followed by incubation at 37°C overnight.

Please replace the third paragraph on page 54, from line 21 to page 55, line 6 with the following:

A48  
3  $\mu$ l of the 12-mer and 2  $\mu$ l of the 8-mer phosphorylated oligonucleotides were added to the cDNA prepared as above in a 300  $\mu$ l reaction mixture containing 6 mM Tris pH 7.5, 6 mM  $MgCl_2$ , 5 mM NaCl, 0.35 mg/ml BSA, 7 mM mercaptoethanol, 0.1 mM ATP, 2 mM DTT, 1 mM spermidine and 400 units T4 DNA ligase (New England BioLabs) at 15° overnight. 10  $\mu$ l of 0.5 M EDTA were added, the reaction was phenol extracted, ethanol precipitated, resuspended in a volume of 100  $\mu$ l and layered on a 5 ml gradient of 5-20% potassium acetate in 1 mM EDTA, 1  $\mu$ g/ml ethidium bromide. The gradient was spun 3 hours at 50,000 rpm (SW55 rotor) and fractionated manually, collecting three approximately 0.5 ml fractions followed by six approximately 0.25 ml fractions in microcentrifuge tubes by means of a butterfly infusion set inserted just above the curve of the tube. Linear polyacrylamide was added to 20  $\mu$ g/ml, the tubes were filled with ethanol, chilled, spun, vortexed and respun as above. The precipitate was washed with 70% ethanol, dried, and resuspended in 10  $\mu$ l. 1  $\mu$ l of the last 6 fractions was run on a gel to determine which fractions to pool, and material less than 1 kb in size was typically discarded. Remaining fractions were pooled and ligated to the vector.

Please replace the second paragraph on page 55, from line 7 to line 18 with the following:

A49  
The complete sequence and derivation of the vector is shown in Figure 5. The vector was prepared for cloning by digestion with BstXI and fractionation on 5-20% potassium acetate gradients as described for the cDNA. The appropriate band was collected by syringe under 300 nm UV light and ethanol precipitated as above. cDNA and vector were titrated in test ligations. Usually 1-2  $\mu$ g of purified vector were used for the cDNA from 4  $\mu$ g of poly A+ RNA. The ligation reactions were composed as described for the adaptor addition above. The ligation reactions were transformed into MC1061/p3 cells made competent as described above. The transformation efficiency for supercoiled vector was  $3-5 \times 10^8$  colonies/ $\mu$ g.

Please replace the third paragraph on page 55, from line 19 to line 25 with the following:

Recovery and characterization of the CD28 clone

ASO Panning of the library was carried out as described herein above, using purified antibody 9.3 (DuPont) at a concentration of 1 µg/ml in the antibody cocktail. The methods used for COS cell transfection, radioimmunoprecipitation, RNA and DNA blot hybridization, and DNA sequencing were all as described herein above.

Please replace the fourth paragraph on page 55, from line 26 to page 56, line 4 with the following:

AS1 To isolate the CD28 cDNA, a large plasmid cDNA library was constructed in a high efficiency expression vector containing an SV40 origin of replication. A preferred version of the vector, containing an M13 origin, is shown in Figures 6A-6D. Three features of the vector make it particularly suitable for this use: (i) the eukaryotic transcription unit allows high level expression in COS cells of coding sequences placed under its control; (ii) The small size and particular arrangement of sequences in the plasmid permit high level replication in COS cells; and (iii) the presence of two identical BstXI sites in inverted orientation and separated by a short replaceable fragment allows the use of an efficient oligonucleotide-based strategy to promote cDNA insertion in the vector.

Please replace the fourth paragraph on page 56, from line 29 to page 57, line 6 with the following:

AS2 Although the cloning scheme of the present invention does not result in a directional insertion of the cDNA, the ability to make large libraries easily, coupled with a powerful selection procedure, makes directional insertion unnecessary. The library construction efficiencies observed according to the present invention, between 0.5 and  $2 \times 10^6$  recombinants per µg of mRNA, with less than 1% background and an insert size greater than 1 kb, compared favorably with those described for phage vectors lambda gt10 ( $7.5 \times 10^5/\mu\text{g}$  of mRNA) and lambda gt11 ( $1.5 \times 10^6/\mu\text{g}$  of mRNA) (Huynh, T., et al., In: DNA Cloning Vol. I, A Practical Approach, Glover, D.M. (ed.), IRL Press, Oxford (1985), pp. 49-78); but the resulting clones were more convenient to manipulate.

Please replace the third paragraph on page 57, from line 24 to page 58, line 2 with the following:

#### Isolation of a CD28 cDNA

AS3  
The CD28 cDNA was isolated from a library of about  $3 \times 10^5$  recombinants prepared from cDNA from 0.8  $\mu$ g of poly A<sup>+</sup> RNA using an earlier version of the protocol described in the Materials and Methods. The library was screened for CD28 (and other surface antigen) cDNA clones by the method outlined above. After the third transfection, COS cells were panned with the 9.3 antibody alone. A Hirt supernatant was prepared from the adherent cells and transformed into E. coli. Plasmid DNA was isolated from eight colonies and transfected individually into COS cell cultures. The presence of the CD28 antigen was detected in three of eight transfected cultures by indirect immunofluorescence. All three plasmid DNAs contained an insert of about 1.5 kb.

Please replace the second paragraph on page 58, from line 3 to line 21 with the following:

#### cDNA sequence analysis

AS4  
The CD28 cDNA encodes a long open reading frame of 220 residues having the typical features of an integral membrane protein (Figures 7A-7B). Removal of a predicted (von Heijne, Nucl. Acids Res. 14:4683-4690 (1986)) N-terminal signal sequence gives a mature protein of 202 residues comprising an extracellular domain with five potential N-linked glycosylation sites (Asn-X-Ser/Thr), a 27-amino acid hydrophobic membrane spanning domain, and a 41-amino acid cytoplasmic domain. Comparison of the amino acid sequence of CD28 with the National Biomedical Research Foundation database (Version 10.0) revealed substantial homology with mouse and rabbit immunoglobulin heavy-chain variable regions over a domain spanning almost the entire extracellular portion of CD28. Within this domain two cysteine residues in the homology blocks Leu-(Ser or Thr)-Cys and Tyr-(Tyr or Phe)-Cys are shared by CD28, CD4, CD8, immunoglobulin heavy- and light-chain variable sequences and related molecules with approximately the same spacing (Maddon et al., Annu. Rev. Biochem. 48:961-997 (1979)).

Please replace the second paragraph on page 61, from line 10 to line 19 with the following:

Preparation of cDNA library and recovery and characterization of CD7 clones

ASS  
Preparation of an HPB-ALL cDNA library in the expression vector pIH3 was carried out as described herein. Panning of the library was carried out according to the method of the present invention, using purified anti-CD7 antibody Leu9 (Becton Dickinson) and antibody 7G5 as ascites fluid was diluted 1:1000. Methods for cell transfection, radioimmunoprecipitation, DNA and RNA blot hybridization and DNA sequencing were all as described herein.

Please replace the third paragraph on page 61, from line 20 to line 35 with the following:

IgM and IgG binding by COS cells transfected with CD7 and CDw32

AS6  
Human IgM, IgG, and IgA antibodies, affinity purified FITC conjugated goat anti-human immunoglobulins antibodies (anti-Ig(G+M+A)), washed and preserved bovine red blood cells, and IgG and IgM fractions of rabbit anti-bovine red blood cell antibodies were purchased from Cooper Biomedical (Malverne, PA). COS cells were transfected by the DEAE Dextran method with cDNAs encoding the CD7, CDw32, and CD28 surface antigens. 48 hours after transfection the cells were washed with PBS/0.5% BSA and incubated with either human IgM, IgG or IgA antibodies at a concentration of 1 µg/ml, at 4°C for 2 hours. Subsequently the cells were washed with PBS/0.5% BSA and incubated for 30 minutes at 4°C with FITC conjugated rabbit anti-human immunoglobulins. After washing the cells were examined with a fluorescence microscope. The experiments were also performed in the presence of 0.1% azide with the same results.

Please replace the second paragraph on page 62, from line 17 to line 26 with the following:

Formation of T cell rosettes with antibody-coated erythrocytes

AS7  
Peripheral blood lymphocytes were obtained from heparinized blood by centrifugation at 4°C over a Ficoll-Hypaque gradient at 400 x g for 30 minutes. Leukocytes at the interface were washed two times with PBS. The leukocytes were adjusted to 10<sup>7</sup> cells/ml in IMDM/10% Fetal Bovine Serum (FBS) and incubated in tissue culture dishes at 37°C for 30 minutes. Nonadherent cells were transferred to new

Got A57  
dishes, and PHA was added to stimulate proliferation of T lymphocytes. On the next day the cells were washed with PBS and placed in fresh IMDM/10%FBS.

Please replace the third paragraph on page 62, from line 27 to page 63, line 2 with the following:

A58  
Rosette assays were performed three days later. Cells were washed with PBS/0.5% BSA, and a 10  $\mu$ l suspension of 2% Ig-coated erythrocytes prepared as described above was added to 10  $\mu$ l of PBS/0.5% BSA containing  $5 \times 10^6$  cells/ml. The mixtures were placed in Falcon round bottom 96 well plates and centrifuged at 150 X g for 15 min at 4°C. After an additional incubation of 45 min at 4°C pellets were resuspended with 10  $\mu$ l of PBS/0.5% BSA, and the rosettes scored by phase contrast microscopy. The experiments were carried out in both the presence and absence of 0.1% sodium azide with no detectable difference.

Please replace the fourth paragraph on page 63, from line 32 to page 64, line 23 with the following:

#### CD7 cDNA sequence analysis

A59  
Both isolates were sequenced by the dideoxynucleotide method. The 1.2 kb cDNA encodes a long open reading frame of 240 residues having the typical features of an integral membrane protein. The initial assignment of the signal sequence cleavage site by the method of von Heijne (Nucl. Acids Res. 14:4683-4690 (1986)) was at the 18th residue. It later was determined, however, that the homology with immunoglobulin variable regions would better predict the mature terminus at residue 26; this assignment would also correlate well with the position of the intron as discussed below and as shown in Figures 8A-8B. Removal of the predicted N-terminal signal sequence gives a mature protein of 215 residues with a predicted molecular mass of 23 kd. In the extracellular domain are two N-linked glycosylation sites (Asn-X-Ser Thr), in agreement with the results of Sutherland et al. (J. Immunol. 133:327-333 (1984)), who also showed the presence of O-linked glycans and covalently associated palmitic acid on the mature protein. In the 27 amino acid hydrophobic membrane spanning domain is a single cysteine residue which may be the site of fatty acylation (Rose et al., Proc. Natl. Acad. Sci. USA 81:2050-2054 (1984); Kaufman et al., J. Biol. Chem. 259:7230-7238 (1984)). The length of the cytoplasmic domain, 39 residues, is in good agreement with the 30-40 amino acids predicted by protease

Out  
59

digestion of the CD7 precursor in rough microsomal membrane fractions (Sutherland et al., J. Immunol. 133:327-333 (1984)).

Please replace the fourth paragraph on page 64, from line 24 to line 33 with the following:

He0

Sequence analysis of the 1.7 kb clone (Figures 8A-8B) revealed the presence of an intron located 121 bp from the 5' end. The 411 bp intron contains stop codons in all three reading frames and is located just downstream of the secretory signal sequence, as is frequently observed for secreted or surface proteins. Both the 5' and 3' ends of the intron conform to the splice donor/acceptor consensus AAG GTRAGA/.../Y<sub>6-11</sub>NYAG A (Mount, Nucl. Acids Res. 10:459-472 (1982)). Because both the 1.2 and 1.7 kb clones express CD7 antigen equally well in COS cells, the intron must be excised in COS cells fairly efficiently.

Please replace the fourth paragraph on page 69, from line 23 to page 70, line 7 with the following:

He1

The nucleotide sequence of the isolated receptor (Figures 9A - 9B) is highly homologous to that of members of the recently isolated murine receptor family, and most closely related to the murine beta<sub>2</sub> receptor by nucleic acid homology. Surprisingly, the murine beta<sub>2</sub> receptor is found on T and B lymphocytes and macrophages, while the alpha receptor is restricted to macrophages; in the human system, CDw32 (shown here to be beta<sub>2</sub>-like) is restricted to macrophages while another Fc receptor (CD16) is found on lymphocytes and macrophages. The human sequence appears to have diverged from the mouse sequence by insertion of approximately 1 kb of DNA a few bases 3' to the junction between the transmembrane and cytoplasmic domains. The junctions of the insertion site do not show obvious relationships to splice donor and acceptor sequences. Comparison of the human and murine peptide sequences showed that the peptide sequence diverges at the end of the transmembrane domain, before the nucleotide sequence diverges, suggesting the existence of a selective pressure favoring the creation of a different cytoplasmic domain.

Please replace the fifth paragraph on page 71, from line 30 to line 34 with the following:

A62  
DNA and RNA blot analysis and hybridization probe preparation were carried out as described. Sequencing was done by the method of Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463 (1977)). The nucleotide sequence of the CD20.4 cDNA is represented in Figures 10A - 10B.

Please replace the first paragraph on page 73, from line 1 to line 34 with the following:

A63  
The amino acid sequence predicted by the cDNA contains 297 residues and has a molecular mass of 33,097 daltons. The sequence contains three major hydrophobic stretches involving residues 51-103, 117-141 and 183-203 (Figures 10A-10B). Two other notable characteristics are the absence of an amino-terminal signal peptide and the presence of a highly charged carboxy-terminal domain. A polyclonal anti-CD20 antibody that recognized the last 18 residues of the carboxy-terminus reacts with lysates of cells expressing CD20 but not with intact cells, suggesting that the CD20 carboxy terminus is located within the cytoplasm. Since there is no amino-terminal signal peptide, it is likely that the amino-terminus is also intracellular, and that the first hydrophobic region acts as an internal membrane insertion signal (Zerial et al., EMBO J. 5:1543 (1986)). The first hydrophobic region is composed of 53 residues and is therefore long enough to span the membrane twice if organized as an alpha helix. Because there are two remaining hydrophobic regions, the intracellular localization of the carboxy-terminus requires that the first hydrophobic domain exit the membrane on the side. Alternatively, the carboxy-terminal antibody may only recognize epitopes exposed by detergent treatment allowing the carboxy-terminus to be extracellular and forcing the first hydrophobic domain to exit the membrane on the extracellular side. The sequence contains 2 potential N-glycosylation sites (Asn-Xaa-Ser/Thr, where Xaa cannot be Pro (Bause, Biochem. J. 209:331 (1983)) at positions 9 and 293, but neither of these is expected to be used if located in intracellular domains of the molecule. The difference in molecular mass between CD20 expressed on COS cells and on B cells is therefore presumably due to O-linked glycosylation, although other forms of post-translational modification are not excluded. If the carboxy-terminus is intracellular, the only extracellular domain would lie between residues 142 and 182. This region is rich in serine and threonine residues which might support O-glycosylation.



Please replace the second paragraph on page 77, from line 3 to line 17 with the following:

AB4 The sequence of the pICAM-1 cDNA insert consists of 1846 nucleotides (Figures 11A - 11C). The predicted peptide sequence of 532 residues has the typical features of a transmembrane protein including a putative signal sequence, which may be cleaved between glycine-25 and asparagine-26 (von Heijne, G., Nucl. Acids Res. 14:4683-4690 (1986)), and a single 25 residue membrane-spanning domain terminating in a short, highly charged cytoplasmic domain. The extracellular domain contains seven potential N-linked glycosylation sites which could adequately explain the difference in size between the deglycosylated precursor (55 kd) and the final product (90-115 kd) (Dustin, M.L., et al., J. Immunol. 137:245-254 (1986)). Differential use of these putative glycosylation sites could also explain the heterogeneous molecular mass of ICAM-1 observed in different cell types (Dustin, M.L., et al., J. Immunol. 137:245-254 (1986)).

Please replace the third paragraph on page 78, from line 20 to line 29 with the following:

AB5 Through its cell adhesion to LFA-1, ICAM can mediate migration of lymphocytes into areas of inflammation. Inhibiting such migration by blocking ICAM binding to LFA-1 could reduce or inhibit inflammation. Such inhibition could be affected by small organic molecules, i.e., drugs, identified in an ICAM streaming assay. Fusion proteins composed of the extracellular domain of ICAM and IgG molecules are suitable for identifying such inhibitors. Likewise, compounds that interfere with ICAM binding to Rhinovirus or *Plasmodium falciparum* can be identified by analogous methods.

Please replace the second paragraph on page 81, from line 10 to line 19 with the following:

Example VIII Isolation and Molecular Cloning of the Human CD19, CD20, CDw32a, CDw32b and CD40 Antigens

AB6 The rapid immunoselection cloning method of the present invention was applied to isolate and clone the CD19, CD20, CDw32a, CDw32b, and CD40 antigens. The nucleotide sequence of CD19 is shown in Figures 12A-12B. The nucleotide sequence of CD20 is shown in Figures 13A - 13B. The

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nucleotide sequence of CDw32a is shown in Figures 14A - 14B. The nucleotide sequence of CDw32b is shown in Figures 15A - 15B. The nucleotide sequence of CD40 is shown in Figure 16.

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Table 1

Page 29

Please replace the third paragraph on page 86, from line 24 to line 30 with the following:

A58 The predicted polypeptide sequences show the typical features of a type I integral membrane protein, and include a short hydrophobic signal sequence, a single 21-residue hydrophobic membrane-spanning domain, and a short, highly charged cytoplasmic domain (Figure 4A). The extracellular portion contains six potential N-linked glycosylation sites and six Cys residues distributed among three C2 set Ig-related domains.

[illegible]

Table 3

Page 32

Table 4  
(SEQ ID NO:26)

1121 CAGTTCTTCT GGGAGNAAAA TGGCAGGCTT CTGGGGAAG AAGCCAGCT GAATTTTGAC TCCATCTCTCC CAGAAGATGC  
Q F F W E K N G R L L G K E S Q L N F D S I S P E D A

1201 TGGGAGTTAC AGCTGCTGGG TGAACAACTC CATAGGACAG ACAGCGTCCA AGGCTGGAC ACTTGAAGTG CTGTATGCAC  
G S Y S C W V N N S I G Q T A S K A W T L E V L Y A P

1281 CCAGGAGGCT GCGTGTGTCC ATGAGCCCGG GGGACCAAGT GATGGAGGGG AAGAGTGCAA CCCTGACCTG TGAGAGCGAC  
R R L R V S M S P G D Q V M E G K S A T L T C E S D

1361 GCCAACCCCTC CCGTCTCCCC CTACACCTGG TTGTACTGGA ATAACCAAAG CCTCCCTTAC CACAGCCAGA AGCTGAGATT  
A N P P V S H Y T W F D W N N Q S L P Y H S Q K L R L

1441 GGAGCCCGTG AAGGTCCAGC ACTCGGTGC CTACTGGTGC CAGGGGACCA ACAGTGTGGG CAAGGGCCGT TCGCCTCTCA  
E P V K V Q H S G A Y W C Q G T N S V G K G R S P L S

1521 GCACCCCTCAC CGTCTACTAT AGCCCGGAGA CCATCGGCAG GCGAGTGGCT GTGGGACTCG GGTCCTGCCT CGCCATCCTC  
T L T V Y Y S P E T I G R R V A V G L G S C L A I L  
=====

1601 ATCTGGCAA TCTGTGGGCT CAAGCTCCAG CGACGTTGGA AGAGGACACA GAGCCAGCAG GGGCTTCAGG AGAATTCACG  
I L A I C G L K L Q R R W K R T Q S Q Q G L Q E N S S  
=====

1681 CGGCCAGAGC TTCTTTGTGA GGAATAAAAA GGTTAGAAGG GCCCCCTCT CTGAAGGCC CCACTCCCTG GGATGCTACA  
G Q S F F V R N K K V R R A P L S E G P H S L G C Y N

1761 ATCCAAATGAT GGAAGATGGC ATTAGCTACA CCACCCCTGG CTTTCCCGAG ATGAACATAC CACGAACCTGG AGATGCAGAG  
P M M E D G I S Y T T L R F P E M N I P R T G D A E

1841 TCCTCAGAGA TGCAGAGACC TCCCCCGGAC TGCATGACA CGGTCACTTA TTCAGCATTG CACAAGCGCC AAGTGGGCAC  
S S E M Q R P P P D C D D T V T Y S A L H K R Q V G T

1921 TATGAGAACG TCATTCCAGA TTTTCCAGAA GATGAGGGA TTCATTACTC AGAGCTGATC CAGTTTGGGG TCGGGGAGCG  
M R T S F Q I F Q K M R G F I T Q S \*

2001 GCCTCAGGCA CAAGAAATG TGGACTATGT GATCCTCAAA CATTGAGACT GGATGGGCTG CAGCAGAGGC ACTGGGGGCA

2081 GCGGGGGCCA GGAAGTCCC CGAGTTT

Table 4 - continued  
(SEQ ID NO:26)

1 ACGCGGAAAC AGGCTTGCAC CCAGACACGA CACCATGCAT CTCCTCGGCC CCTGGCTCCT GCTCCTGGTT CTAGAACTACT  
M H L L G P W L L L L V L E Y L

81 TGGCTTTCTC TGACTCAAGT AAATGGGTTT TTGAGCACCC TGAAACCCCTC TACGCCCTGGG AGGGGGCCTG CGTCFGGATC  
A F S D S S K W V F E H P E T L Y A W E G A C V W I

161 CCCTGCACTT ACAGAGCCCT AGATGGTGAC CTGGMAAGCT TCATCCTGTT CCACAACTCT GAGTATAACA AGAACACCTC  
P C T Y R A L D G D L E S F I L F H N P E Y N K N T S

241 GAAAGTTTGAT GGGACAAAGAC TCTATGAAAG CACMAAGGAT GGAAGGTTT CTTCTGAGCA GAAAGGGGTG CAATTCTGG  
K F D G T R L Y E S T K D G K V P S E Q K R V Q F L G

321 GAGACAAAGAA TAAGAACTGC ACACTGAGTA TCCACCCGGT GACCTCAAT GACAGTGGTC AGCTGGGGCT GAGGATGG  
D K N K N C T L S I H P V H L N D S G Q L G L R M E

401 TCCAAAGACTG AGAAATGGAT GGAACGAATA CACCTCAATG TCTCTGMAAG GCCTTTTCCA CCTCATATCC AGCTCCCTCC  
S K T E K W M E R I H L N V S E R P F P P H I Q L P P

481 AGAAATTCAA GAGTCCCAGG AAGTCACCTT GACCTGCTTG GACCTTTCT CTGCTATGG GTATCCGATC CAATTGCAAT  
E I Q E S Q E V T L T C L L N F S C Y G Y P I Q L Q W

561 GGCTCCTAGA GGGGGTTCCA ATGAGGCAGG CTGCTGTAC CTGACCTCC TTGACCATCA AGTCTGTCTT CACCCGGAGC  
L L E G V P M R Q A A V T S T S L T I K S V F T R S

641 GAGCTCAAGT TCTCCCAACA GTGGAGTCAC CATGGGAAGA TTGTGACCTG CCAGCTTCAG GATGCAGATG GGAAGTTCCT  
E L K F S P Q W S H H G K I V T C Q L Q D A D G K F L

721 CTCCAAATGAC ACGGTGCAGC TGAAAGTGAA GCATCCTCCC AAGAAAGTGA CCACAGTGAT TCNAAACCCC ATGCCGATTC  
S N D T V Q L N V K H P P K K V T T V I Q N P M P I R

801 GAGNAGGAGA CACAGTGACC CTTTCCTGTA ACTACAAATC CAGTAACCCC AGTGTACCC GGTATGAATG GAAACCCCA  
E G D T V T L S C N Y N S S N P S V T R Y E W K P H

881 GCGCCTGGG AGGAGCCATC GCTTGGGTG CTGAAGATCC AAAAGCTTG CTGGGACAAAC ACAACCATCG CCTGGCGCAGC  
G A W E E P S L G V L K I Q N V G W D N T T I A C A A

961 TTGTAATAGT TGGTGCTCGT GGGCCTCCCC TGTCGCCCTG AATGTCCAGT ATGCCCCCG AGACGTGAGG GTCCGGAAAA  
C N S W C S W A S P V A L N V Q Y A P R D V R V R K I

1041 TCNAGCCCTT TTCCGAGATT CACTCTGGAA ACTCGGTCAG CCTCCAATGT GACTTCTCAA GCAGCCACCC CANAGAAATC  
K P L S E I H S G N S V S L Q C D F S S S H P K E V



Please replace the fourth paragraph on page 94, from line 25 to page 95, line 3 with the following:

A72 Example XIII The Isolation and Molecular Cloning of cDNA Encoding for T Lymphocyte-specific CD27 Antigen

A cDNA clone encoding CD27 was obtained from human T lymphocyte cDNA transferred into COS cells and immunoselected by the method of the present invention. RNA was extracted from the mononuclear cells derived from a unit of blood, after four days of culture in medium containing 1 µg/ml phytohemagglutinin (PHA), using guanidium thiocyanate. The total RNA was poly-A selected. cDNA was made and cloned into CDM8, transfected into COS cells and the CD27 cDNA was immunoselected with monoclonal antibodies OKT18a and CLB-9F4 (provided as described in Seed and Aruffo Proc. Natl. Acad. Sci. USA 84:8573-8577 (1987); and Aruffo and Seed Proc. Natl. Acad. Sci. USA 84:3365-3369 (1987)). The vector contained a 1.2 kb cDNA insert.

Table 5  
(SEQ ID NO:28)

1 GGGGTGCAAA GAAGAGACAG CAGCGCCCGAG CTTGGAGGTG CTAACCTCCAG AGGCCAGCAT CAGCAACTGG GCACAGAAAG  
 81 GAGCGGCGCTG GGCAGGGGACC <sup>1</sup>ATGGCACGGC CACATCCCTG GTGGCTGTGC GTTCTGGGGA CCCTGGTGGG GCTCTCAGCT  
 161 ACTCCAGCCC CCAAGAGCTG CCCAGAGAGG CACTACTGGG CTCAGGGAAA GCTGTGCTGC CAGATGTGTG AGCCAGGAAC  
 241 ATTCCCTCGTG AAGGACTGTG ACCAGCATAG AAGGCTGCT CAGTGTGATC CTTGCATACC GGGGGTCTCC TTCTCTCCTG  
 321 ACCACCACAC CCGGCCCCAC TGTGAGAGCT GTCGGCACTG TAACTCTGGT L L V R N C T I T A  
 401 AATGCTGAGT GTGCTGTGTC CAATGGCTGG CAGTGCAGGG ACAAGGAGTG CACCGAGTGT GATCCTCTTC CAAACCCTTC  
 481 GCTGACCGCT CGGTCGTCTC AGGCCCTGAG CCCACACCCCT CAGCCCCACCC ACTTACCTTA TGTCACTGAG ATGCTGGAGG  
 561 CCAGGACAGC TGGGCACATG CAGACTCTGG CTGACTTCAG GCAGCTGCCT GCCCGGACTC TCTCTACCCA CTGGCCACCC  
 641 CAAAGATCCC TGTGCAGCTC CGATTCTTATT CGCATCCTTG TGATCTTCTC TGGAAATGTTT CTTGTCTTCA CCCTGGCCGG  
 721 GGCCTGTTC CTCCATCMAC GAAGGAANTA TAGATCAAAC AAAGGAGAAA GTCCCTGTGA GCCTGCAGAG CCTTGTCTGTT  
 801 ACAGCTGCCC CAGGGAGGAG GAGGGCAGCA CCATCCCAT I P I Q E D Y R K P E P A C S P  
 881 TGAGCCAGCA CCTGCGGTAG CTGCACACTA GCCCTGGCCT CCACCCCCAC CCGCGCGACC ATCCAAAGGA GAGTGAGACC  
 961 TGGCAGCCAC AACTGCAGTC CCATCCTCTT GTCAGGGCCC TTTCTCTGTGT ACACGTGACA GAGTGCCTTT TCGAGACTGG  
 1041 CAGGGACGAG GACAAATATG GATGAGGTGG AGAGTGGGMA GCAGGAGCCC AGCCAGCTGC GCGCGCGTGC AGGAGGGCGG  
 1121 GGGCTCTGGT TGTAAAGGCAC ACTTCTCTGCT GCGAAAGACC CACATGCTAC AAGACGGGCA AATATAAGTG ACAGATGACC

Table 6  
(SEQ ID NO:30)

|     |            |             |             |            |            |             |            |               |
|-----|------------|-------------|-------------|------------|------------|-------------|------------|---------------|
| 1   | CTCCCTTTGG | GCAAGGACCT  | GAGACCCCTTG | TGCTAAGTCA | AGAGGCTCAA | TGGGCTGCAG  | AAGAACTAGA | GAAGGACCAA    |
|     |            |             |             |            |            | M           | G C R      | R T R E G P S |
| 81  | GCAAGGCCAT | GATATTTCCA  | TGGAATGTC   | AGAGCACCCA | GAGGGACTTA | TGGAACATCT  | TCAAGTTGTG | GGGTGGACA     |
|     | K A M      | I F P       | W K C Q     | S T Q      | R D L      | W N I F     | K L W      | G W T         |
| 161 | ATGCTCTGTT | GTGATTTCCCT | GGCACATCAT  | GGAACCGACT | GCTGGACTTA | CCATTATTCT  | GAANAACCCA | TGAAGTGGCA    |
|     | M L C C    | D F L       | A H H       | G T D C    | W T Y      | H Y S       | E K P M    | N W Q         |
| 241 | AAGGGCTAGA | AGATTCTGCC  | GAGACAAATTA | CACAGATTTA | GTTGCCCTAC | AAAACAAGGC  | GGAATTTGAG | TATCTGGAGA    |
|     | R A R      | R F C R     | D N Y       | T D L      | V A I Q    | N K A       | E I E      | Y L E K       |
| 321 | AGACTCTGCC | TTTCAGTCGT  | TCTTACTACT  | GGTAGGAAT  | CCGGAAGATA | GGAGGAATAT  | GGACGTGGGT | GGGAACCAAC    |
|     | T L P      | F S R       | S Y Y W     | I G I      | R K I      | G G I W     | T W V      | G T N         |
| 401 | AAATCTCTCA | CTGAAGAGC   | AGAGAACTGG  | GGAGATGGTG | AGCCCAACAA | CAAGNAGAAC  | AAGGAGGACT | GCGTGGAGAT    |
|     | K S L T    | E E A       | E N W       | G D G E    | P N N      | K K N       | K E D C    | V E I         |
| 481 | CTATATCAAG | AGAAACAAAG  | ATGCAGGCCAA | ATGGAACGAT | GACGCCTGCC | ACAAACTAAA  | GGCAGCCCTC | TGTTACACAG    |
|     | Y I K      | R N K D     | A G K       | W N D      | D A C H    | K L K       | A A L      | C Y T A       |
| 561 | CTTCTTGCCA | GCCCTGGTCA  | TGCAGTGGCC  | ATGGAGAATG | TGTAGAAATC | ATCAATAATT  | ACACCTGCNA | CTGTGATGTG    |
|     | S C Q      | P W S       | C S G H     | G E C      | V E I      | I N N Y     | T C N      | C D V         |
| 641 | GGGTACTATG | GGCCCCCAGT  | TCAGTTTGTG  | ATTCAGTGTG | AGCCTTTGGA | GGCCCCCAGAG | CTGGGTACCA | TGGACTGTAC    |
|     | G Y Y G    | P Q C       | Q F V       | I Q C E    | P L E      | A P E       | L G T M    | D C T         |
| 721 | TCACTCTTTG | GGAAACTTCA  | GCTTCAGCTC  | ACAGTGTGCC | TTCAGCTGCT | CTGAAGGAAC  | AAACTTAACT | GGGATTGAAG    |
|     | H S L      | G N F S     | F S S       | Q C A      | F S C S    | E G T       | N L T      | G I E E       |
| 801 | AAACCACCTG | TGGACCATT   | GGAAACTGGT  | CATCTCCAGA | ACCAACCTGT | CAAGTGATTC  | AGTGTGAGCC | TCTATCAGCA    |
|     | T T C      | G P F       | G N W S     | S P E      | P T C      | Q V I Q     | C E P      | L S A         |
| 881 | CCAGATTGG  | GGATCATGAA  | CTGTAGCCAT  | CCCCCTGGCA | GCTTCAGCTT | TACCTCTGCA  | TGTACCTTCA | TCTGCTCAGA    |
|     | P D L G    | I M N       | C S H       | P L A S    | F S F      | T S A       | C T F I    | C S E         |

--CHO--

(1)

--C HO--

--CH O--

--CHO--

--CHO--

--CHO--

Cont  
A74

Table 6 - continued  
(SEQ ID NO:30)

961 AGGAACTGAG TTAATTGGGA AGAAGAAAAC CATTTGTGAA TCATCTGGAA TCTGGTCAAA TCCTAGTCCA ATATGTCAAA  
G T E L I G K K K T I C E S S G I W S N P S P I C Q K  
CHIO--

1041 AATTGGACAA AAGTTTCTCA ATGATTAAAGG AGGGTGATTA TAACCCCTC TTCATTCCAG TGGCAGTCAT GGTACTGCA  
L D K S F S M I K E G D Y N P L F I P V A V M V T A  
=====

1121 TTCTCTGGGT TGGCATTTAT CATTGGCTG GCAAGGAGAT TAAAAAAGG CAAAGAAATCC AAGAGAAGTA TGAATGACCC  
F S G L A F I I W L A R R L K K G K K S K R S M N D P  
=====

1201 ATATTAAATC GCCCTTGGTG AAAGAAAATT CTTGGANTAC TAAAAATCAT GAGATCCTTT AAATCCTTCC ATGAAAACGTT  
Y \*

1281 TTGTGTGGTG GCACCTCCTA CGTCAAACAT GAAGTGTGTT TCCTTCAGTG CATCTGGGA GATTCTTACC TGACCAACAG  
=====

1361 TTCCCTTCAGC TTCCATTTCG CCCCTCATTT ATCCCTCAAC CCCAGGCCA CAGGTGTTTA TACAGCTCAG CTTTTGTCT  
=====

1441 TTTCTGAGGA GAAACAAATA AGACCATAAA GGGAAAGGAT TCATGTGGAA TATAAAGATG GCTGACTTGG CTCTTTCTTG  
F L  
(293)

1521 ACTCTTGTTT TCAGTTTCAA TTCAGTGCTG TACTTGATGA CAGACACTTC TAAATGAAGT GCAAAATTGA TACATATGTG  
T L V F S F N S V L Y L M T D T S K \*

1601 AATATGGACT CAGTTTCTT GCAGATCAAA TTTACAGTCG TCTTCTGTAT ACTGTGGAGG TACACTCTTA TAGAAAGTTC  
=====

1681 AAAAAAGTCTA CGCTCTCCTT TCTTTCTAAC TCCAGTGAAG TAATGGGGTC CTGCTCAAGT TGAAGAGATC CTATTTCAC  
=====

1761 TGTAGCCTCG CCGTCTGTGA ATTGGACCAT CCTATTAAAC TGGCTTCAGC CTCCCCACCT TCTTCAGCCA CCTCTCTTTT  
=====

1841 TCAGTTGGCT GACTTCCACA CCTAGCATCT CATGAGTGCC AAGCAAAAGG AGAGAAGAGA GAAATAGCCT GCGCTGTTTT  
=====

1921 TTAGTTGGG GGTTTGCTG TTTCCCTTTTA TGAGACCCAT TCCTATTCT TATAGTCAAT GTTCTTTTA TCACGATATT  
=====

Cont  
A74

Table 6 - continued  
(SEQ ID NO:30)

2001 ATTAGTAAGA AACATCACT GAAATGCTAG CTGCAAGTGA CATCTCTTTG ATGTCATATG GAAAGAGTTAA AACAGGGTGG  
2081 GAAATTCCCTT GATTCACAAAT GAAATGCTCT CTTTCCCTT GCGCCGAGAC CTTTATCCG ACTTACCTAG ATTCTACATA  
2161 TTCTTTAAAT TTCATCTCAG GCTCCCTCA ACCCCACCAC TTCTTTTATA ACTAGTCTT TACTAAATCCA ACCCATGATG  
2241 AGCTCCCTCTT CCTGGCTTCT TACTGAAAGG TTACCCCTGTA ACATGCAATT TTGCATTGTA ATAAAGCCTG CTTTTTAAAGT  
2321 GTTAAAAAAA AAAAAAAAAA AAAAAAAAAA

A75

A75

Cont  
A75

Table 7 - continued  
(SEQ ID NO:31)

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--C HO--
961  TCTTGCAGTT TGCATTGCAG TCAACAGTCG AAGAAGGTGT GGCAGAGAAGA AAAAGCTAGT GATCAACAGT GGCNAATGGAG
    L A V C I A V N S R R R C G Q K K K L V I N S G N G A
=====
1041 CTGTGGAGGA CAGAAAGCCA AGTGGACTCA ACGGAGAGGC CAGCAGTCT CAGGNAATGG TGCATTGGT GAACAAAGGAG
    V E D R K P S G L N G E A S K S Q E M V H L V N K E
1121 TCGTCAGAAA CTCCAGACCA GTTATGACA GCTGATGAGA CAAGGAACCT GCAGAAATGTG GACATGAAGA TTGGGGTGTA
    S S E T P D Q F M T A D E T R N L Q N V D M K I G V *
1201 ACACCTACAC CATTATCTTG GAAAGNAACA ACCGTTGTAA ACNTAACCAT TACAGGGAGC TGGGACACTT AACAGATGCA
1281 ATGTGCTACT GATTGTTTCA TTGCGAATCT TTTTAGCAT AAAATTTTCT ACTCTTTTGT TAAAAAAAAA AAAA 1354

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A26

Table 8  
(SEQ ID NO:33)

|     |            |             |            |             |             |             |            |             |
|-----|------------|-------------|------------|-------------|-------------|-------------|------------|-------------|
| 1   | CCAGCCTCTG | CCAGGTTCCG  | TCCGCCATCC | TCGTCCCGTC  | CTCCGCCGGC  | CCCTGCCCCG  | CGCCACAGGA | TCCTCCAGCT  |
| 81  | CCTTTCGCC  | GCGCCCTCCG  | TTCGCTCCGG | ACACCATGGA  | CAAGTTTGG   | TGGCAGCGAG  | CCTGGGGACT | CTGCCCTCGTG |
|     |            |             |            | M D         | K F W       | W H A       | A W G L    | C L V       |
| 161 | CCGCTGAGCC | TGGCGCAGAT  | CGATTGGAAT | ATAACCTGCC  | GCTTTGCAGG  | TGTATTCCAC  | GTGGAGAAAA | ATGGTCGCTA  |
|     | P L S L    | A Q I       | D L N      | I T C R     | F A G       | V F H       | V E K N    | G R Y       |
|     |            |             |            |             |             |             |            | --C HO--    |
| 241 | CAGCATCTCT | CGGACGGAGG  | CCGCTGACCT | CTGCAAGGCT  | TTCNAATAGCA | CCTTGCCCCAC | ANTGGCCCG  | ATGGAGAAAG  |
|     | S I S      | R T E A     | A D L      | C K A       | F N S       | T L P       | T M A Q    | M E K A     |
| 321 | CTCTGAGCAT | CGGATTGAG   | ACCTGCAGGT | ATGGCTTCAT  | AGAAAGGCAT  | GTGGTGATTC  | CCCGGATCCA | CCCCAACTCC  |
|     | L S I      | G F E       | T C R Y    | G F I       | E G H       | V V I P     | R I H      | P N S       |
|     |            |             |            |             |             |             |            | --CHO--     |
| 401 | ATCTGTGCAG | CAAAACAACAC | AGGGGTGTAC | ATCCTCACAT  | ACAACACCTC  | CCAGTATGAC  | ACATATTGCT | TCAATGCTTC  |
|     | I C A A    | N N T       | G V Y      | I L T Y     | N T S       | Q Y D       | T Y C F    | N A S       |
|     |            |             |            |             |             |             |            | --CHO--     |
| 481 | AGCTCCACCT | GAAGAAGATT  | GTACATCAGT | CACAGACCTG  | CCCAATGCCT  | TTGATGGACC  | AATTACCATA | ACTATTGTTA  |
|     | A P P      | E E D C     | T S V      | T D L       | P N A F     | D G P       | I T I      | T I V N     |
|     |            |             |            |             |             |             |            | --CHO--     |
| 561 | ACCGTGATGG | CACCCGCTAT  | GTCCAGAAAG | GAGAAATACAG | AACGAATCCT  | GAAGACATCT  | ACCCACGCAA | CCCTACTGAT  |
|     | R D G      | T R Y       | V Q K G    | E Y R       | T N P       | E D I Y     | P S N      | P T D       |
|     |            |             |            |             |             |             |            | --CHO--     |
| 641 | GATGACGTGA | GCAGCGGCTC  | CTCCAGTGAA | AGGAGCAGCA  | CTTCAGGAGG  | TTACATCTTT  | TACACCTTTT | CTACTGTACA  |
|     | D D V S    | S G S       | S S E      | R S S T     | S G G       | Y I F       | Y T F S    | T V H       |
|     |            |             |            |             |             |             |            | ----        |
| 721 | CCCCATCCCA | GACGAAGACA  | GTCCCTGGAT | CACCGACAGC  | ACAGACAGAA  | TCCCTCGTAC  | CAATATGGAC | TCCAGTCATA  |
|     | P I P      | D E D S     | P W I      | T D S       | T D R I     | P R T       | N M D      | S S H S     |
|     |            |             |            |             |             |             |            | --CHO--     |
| 801 | GTACAACGCT | TCAGCCTACT  | GCAATCCNA  | ACACAGGTTT  | GGTGGAAAGAT | TTGGACAGGA  | CAGGACCTCT | TTCAATGACA  |
|     | T T L      | Q P T       | A N P N    | T G L       | V E D       | L D R T     | G P L      | S M T       |
|     |            |             |            |             |             |             |            | --CHO--     |
| 881 | ACGCAGCAGA | GTAATTCTCA  | GAGCTTCTCT | ACATCACATG  | AAGGCTTGGA  | AGAAGTAA    | GACCATCCAA | CAACTTCTAC  |
|     | T Q Q S    | N S Q       | S F S      | T S H E     | G L E       | E D K       | D H P T    | T S T       |
|     |            |             |            |             |             |             |            | --CHO--     |
| 961 | TCTGACATCA | AGCAATAGGA  | ATGATGTCAC | AGGTGGNAGA  | AGAGACCCAA  | ATCATTTCTGA | AGGCTCAACT | CATTACTG    |
|     | L T S      | S N R N     | D V T      | G G R       | R D P N     | H S E       | G S T      | H L L E     |



Cont  
A76

Table 8 - continued  
(SEQ ID NO:33)

1041 AAGGTTATAC CTCTCATATAC CCACACACAGA AGGAAGCAG GACCTTCATC CCAGTGACCT CAGCTAAGAC TGGGTCCTTT  
G Y T S H Y P H T K E S R T F I P V T S A K T G S F  
1121 GGAGTTACTG CAGTTACTGT TGGAGATTCC AACTCTAATG TCAATCGTTC CTATACAGGA GACCAAGACA CATTCCACCC  
G V T A V T V G D S N S N V N R S L S G D Q D T F H P  
1201 CAGTGGGGGG TCCCATACCA CTCATGGATC TGAATCAGAT GGACACTCAC ATGGGAGTCA AGAAGGTGGA GCAACACAAA  
S G G S H T T H G S E S D G H S H G S Q E G G A N T T  
1281 CCTCTGGTCC TATAAGGACA CCCCNAATTC CAGAATGGCT GATCATCTTG GCATCCCTCT TGGCCTTGGC TTTGATTCTT  
S G P I R T P Q I P E W L I I L A S L L A L A L I L  
-----  
=====

1361 GCAGTTTGCA TTGCAGTCAA CAGTCGAAGA AGGTGTGGG AGAAGAAAA GCTAGTGATC AACAGTGGCA ATGGAGCTGT  
A V C I A V N S R R R C G Q K K K L V I N S G N G A V  
=====

1441 GGAGGACAGA NAGCCAAAGTG GACTCAACGG AGAGGCCAGC AAGTCTCAGG AAATGGTGCA TTTGGTGAAC AAGGAGTCGT  
E D R K P S G L N G E A S K S Q E M V H L V N K E S S  
1521 CAGAAACTCC AGACCAAGTTT ATGACAGCTG ATGAGACAAG GAACCTGCAG AATGTGGACA TGAAGATTGG GGTGTAACAC  
E T P D Q F M T A D E T R N L Q N V D M K I G V \*  
1601 CTACACCATT ATCTTGGAAA GAAACAACGT TGGAAACATA ACCATPACAG GGGAGCTGGG ACACCTTAACA GATGCAATGT  
1681 GCTACTGATT GTTTCATTTC GAATCTATAA TAGCATAAAA TTTTCTACTC TTTTGTGTTT TTTGTGTTTG TTCTTTAAG  
1761 TCAGGTCCAA TTTGTAAAAA CAGCATTGCT TTCTGAAATT AGGCCCAAT TAATAATCAG CAAGAATTTT GATCGTTTCA  
1841 GTTCCCCACT TGGAGGCCCT TCATCCCTCG GGTGTGCTAT GGATGGCTTC TAAACAAAAA CTACCACATA GTTATTCCTG  
1921 ATCGCCCAACC TTGCCCCCCA CCAGCTAAGG ACATTTCCAG GGTAAATAGG GCCTGGTCCCT GGGAGGAAAT TTGAATGGGT  
2001 CATTTTGGCC TTCCATTAGC CTAATCCCTG GGCATTGCTT TCCACTGAGG TTGGGGGCTG GGGTGTACTA GTTACACATC  
2081 TTCAACAGAC CCCCTCTAGA AATTTTTCAG ATGCTTCTGG GAGACACCCA AAGGTAAGT CTATTTATCT GTAGTAAACT  
2161 ATTTATCTGT GTTTTGGAAA TATTAAACCC TGGATCAGTC CTTTATTCA GTATAATTTT TTAAGTTAC TTTGTCAGAG  
2241 GCACAAAAAG GGTTAATACT GATTCATAAT AATATCTGT ACCTTCTCG AAAAAAAA AAAAAAAA

Table 9  
(SEQ ID NO:35)

|     |   |         |
|-----|---|---------|
| 1   | CTCAAGGATA ATCACTAAAT TCTGCCGAAA GGAAGGGA ACGGTGCTG GAAAAGGGCA AGAATATCAC GGCATGGGCA      | M G M   |
| 81  | TGAGTAGCTT GAAACTGCTG AAGTATGTCC TGTTTTTCTT CAACTTGCTC TTTTGGATCT GTGGCTGCTG CATTTTGGGC   | I L G   |
| 161 | TTTGGGATCT ACCTGCTGAT CCACAACAAC TTCCGGAGTGC TCTTCCATAA CCTCCCTCTG L T L G N V F          | =====   |
| 241 | TGTCATCGTG GGCTCTATTA TCATGGTAGT TGCCTTCCCTG GGCTGCATGG GCTCTATCAA GGAACAACAAG TGTCTGCTTA | =====   |
| 321 | TGTCGTTCTT CATCCTGCTG CTGATTATCC TCCTTGCTGA GGAGACCTTG GCCATCCTGC TCTTTGTATA TGAACAGAAAG  | E Q K   |
| 401 | CTGAATGAGT ATGTGGCTAA GGGTCTGACC GACAGCATCC ACCGTACCA CTCAGACAAT AGCACCNAAG CAGCGTGGGA    | A W D   |
| 481 | CTCCATCCAG TCATTCTGTC AGTGTGTGG TATAAATGGC ACAGTGATT GGACCACTGG CCCACCAGCA TCTTGCCCTT     | S C P S |
| 561 | CAGATCGAAA AGTGGAGGGT TGCTATGCGA AAGCAAGACT GTGGTTTCAT TCCAAATTCC TGTATATCGG NATCATCACC   | I I T   |
| 641 | ATCTGTGTAT GTGTGATTGA GGTGTGGGG ATGTCCTTG CACTGACCCT GAACGTGCCAG ATTGACNAAA CCAGCCAGAG    | S Q T   |
| 721 | CATAGGGCTA TGATCTGCAG TAGTTCTGTG GTGAAGAGAC TTGTTTCATC TCCGGAAATG CAAMACCATT TATAGCATGA   | =====   |
| 801 | AGCCCTACAT GATCACTGCA GGATGATCCT CCTCCCATCC TTTCCCTTTT TAGGTCCCTG TCTTATACAA CCAGAGAAAGT  | =====   |
| 881 | GGGTGTTGGC CAGGCACATC CCATCTCAGG CAGCAAGACA ATCTTTCAT CACTGACGGC AGCAGCCATG TCTCTCAAG     | =====   |

Table 9 - continued  
(SEQ ID NO:35)

|      |            |            |            |            |            |            |             |             |
|------|------------|------------|------------|------------|------------|------------|-------------|-------------|
| 961  | TGGTGAAACT | AATATCTGAG | CATCTTTTAG | ACAAGAGAGG | CAAAAGACAA | CTGGATTTAA | TGGCCCCAACA | TCAAAAGGGTG |
|      |            |            |            |            |            | """"       |             |             |
| 1041 | AACCCAGGAT | ATGAATTTT  | GCATCTTCCC | ATTGTCGAAT | TAGTCTCCAG | CCTCTAAATA | ATGCCCAGTC  | TTCTCCCCAA  |
| 1121 | AGTCAAGCAA | GAGACTAGTT | GAAGGGAGTT | CTGGGGCCAG | GCTCAGTGG  | CCATTGTCAC | AACCCCTCTGT | TTCTCTTTGA  |
| 1201 | CTAAGTGCCC | TGGCTACAGG | AATTACACAG | TTCTCTTTCT | CCAAAGGGCA | AGATCTCAAT | TCAATTTCTT  | TATTAGAGGG  |
| 1281 | CCTTATTGAT | GTGTTCTAAG | TCCTTCCAGA | AAAAAACTAT | CCAGTGATTT | ATATCCTGAT | TTCAACCAGT  | CACCTAGCTG  |
|      |            |            |            |            | """"       |            |             |             |
| 1361 | ATAATCACAG | TAAAGAGACT | TCTGGTATTA | TCTCTCTATC | AGATAAGATT | TTGTTAATGT | ACTATTTTAC  | TCTTCAATAA  |
|      |            |            |            |            |            |            |             | -----       |
| 1441 | ATAAAACAGT | TT         | 1452       |            |            |            |             |             |

Drawings:

Figures 1, 2, 4, 6, 7, 8, 9, 11, 12, and 13 have been relabeled to be consistent with the Specification. For example, Figure 1-1 is now Figure 1A and Figure 1-2 is Figure 1B etc.

Figure 15, 16, and 17 have been relabeled as 14A, 14B, 15A, 15B, and 16, respectively. These amendments were necessary to correct inadvertent typographical error, i.e., Figure 14 did not exist in the as-filed Specification.